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एलगी स्पाइरुलिना, पशुआहार ग्रेड – विशिष्टि

Indian Standard

ALGA *SPIRULINA*, FEED GRADE — SPECIFICATION

UDC 636·087·74 ALG

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

June 1992

Price Group 3

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Livestock Feeds Sectional Committee had been approved by the Food and Agriculture Division Council.

Use of algal proteins has been suggested for many years as a means to alleviate protein shortage. Algae have also been used in animal feeds generally and in specialised aqua-feeds in particular as a non-conventional protein source. *Spirulina*, a blue green alga (cyanobacterium), is characterised by high content of protein, β-carotene, B-complex vitamins, particularly cyanocobalamine and gamma-linolenic acid. The amino acid pattern of this protein shows it to be of reasonably good quality and it may be added to enrich various feed formulations. The percentage of essential amino acids in *Spirulina platensis* is reported as lysine 4·8 percent, histidine 1·8 percent, arginine 6·5 percent, threonine 6·2 percent, methionine 2·5 percent, leucine 7·1 percent, isoleucine 6·7 percent and phenylalanine 4.9 percent. In view of high nutrient content besides proteins, *Spirulina* is marketed internationally in natural and health foods, in specialised animal feeds and also in cosmetics. This alga has also been approved by FDA of the United States for being used a natural food. *Spirulina* of species *platensis maxima* and *fusiformis* are commonly used for the commercial production of feed grade alga. This standard is therefore, being formulated to ensure production of *Spirulina* of certain minimum quality.

Feed grade alga *Spirulina* is produced with suitable nutrient medium with chemicals or in diluted and treated effluents from biogas plants or domestic sewage. Though there are variations in the details of the manufacturing processes, the production of feed grade alga basically involves the following steps:

a) Selection of suitable strains of alga;
b) Preparation of nutrient medium and specified culture conditions;
c) Cultivation of selected alga in open culture ponds under defined conditions;
d) Separation of grown alga by filtration and washing to get rid of residual nutrients; and
e) Suitable drying to flakes or fine powder.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

ALGA *SPIRULINA*, FEED GRADE — SPECIFICATION

1 SCOPE

1.1 This standard prescribes the requirements and methods of sampling and test for feed grade alga *Spirulina*.

2 REFERENCES

2.1 The Indian Standards listed in Annex A are necessary adjuncts to this standard.

3 TERMINOLOGY

3.1 Acceptance Tests

Tests carried on samples selected from the lot for the purpose of acceptance of the lot.

3.2 Type Tests

Tests to approve the design and micro nutrient quality of the product at least at the beginning of marketing or certification and conducted periodically thereafter, to check the product quality or whenever the formulation is changed.

4 REQUIREMENTS

4.1 Description

The material shall be in the form of dried powder, granules or flakes. It shall be of uniform dark blue-green colour and shall have the characteristic taste and odour (mild resembling dried greens) of good quality alga, free from any unpleasant musty or putrid smell. It shall be dry and free from lumps and from extraneous matter, added colour and deleterious substances. It shall also be free from rodent contamination, visible mould growth, insect infestation and toxic materials and heavy metals like lead, copper, mercury and arsenic. Pesticide residues shall be within permissible limits.

NOTE — The appearance, taste and odour of alga shall be determined by sensory tests.

4.2 Hygienic Conditions

The material shall be manufactured in the premises maintained under hygienic conditions. If effluent is used proper dilution is to be made and the medium should not contain toxic substances.

4.3 The material shall also comply with the requirements given in Tables 1 and 2.

4.4 Other Algal Contamination

The alga culture shall be tested periodically for absence of toxic algal forms by a bioassay procedure (*see* Annex B).

5 PACKING

5.1 The material shall be packed in clean, sound and opaque HDPE bags or in any other suitable containers, in such a manner as to prevent absorption of extraneous moisture and deterioration during storage.

6 MARKING

6.1 Each bag/container shall be suitably marked to give the following particulars:

a) Name of the material and trade-mark, if any;
b) Name and address of the manufacturer;
c) Date of manufacture;
d) Batch or code number;
e) Nutrients supplied by 100 g;
f) Net mass in g or kg; and
g) Any other details required under the Standards of Weights and Measures (Packaged Commodities) Rules, 1977.

6.1.1 Instructions for use and storage shall be printed on the bag/container as given in Annex C.

7 SAMPLING

7.1 The method of drawing representative samples of the material and the criteria for conformity shall be as prescribed in Annex D.

8 TESTS

8.1 Tests shall be carried out as prescribed in **4.4** and col 4 of Tables 1, 2 and 3.

Table 1 Requirements for Alga *Spirulina*, Feed Grade

(*Clause* 4.3)

Sl No. (1)	Characteristics (2)	Requirement (3)	Method of Test, Ref to (4)
i)	Moisture, percent by mass, *Max*	9·0	4 of IS 7874 (Part 1) : 1975
ii)	Crude fibre (on dry basis), percent by mass, *Max*	10·5	8 of IS 7874 (Part 1) : 1975
iii)	Total ash (on dry basis), percent by mass, *Max*	12·0	9 of IS 7874 (Part 1) : 1975
iv)	Acid insoluble ash (on dry basis), percent by mass, *Max*	2·0	10 of IS 7874 (Part 1) : 1975
v)	Protein (on dry basis), percent by mass, *Min*	50·0	5 of IS 7874 (Part 1) : 1975
vi)	Total fat, percent by mass, *Min*	6·0	7 of IS 7874 (Part 1) : 1975
vii)	Lead, mg/kg, *Max*	6·0	K of IS 1679 : 1960
viii)	Arsenic, mg/kg, *Max*	2·0	J of IS 1679 : 1960
ix)	Mercury, mg/kg, *Max*	0·2	H of IS 9808 : 1981
x)	Total bacterial count/g, *Max*	90000	IS 5402 : 1969
xi)	Coliform bacteria count/g, *Max*	20	IS 5401 : 1969
xii)	*Salmonella* sp. (in one g)	Absent	IS 5887 (Part 3) : 1976
xiii)	*Shigella* (in one g)	Absent	IS 5887 (Part 3) : 1976
xiv)	*E. coli* (in one g)	Absent	IS 5887 (Part 1) : 1976

Table 2 Vitamins and Minerals Requirement per 100 Grams

(*Clause* 4.3)

Sl No. (1)	Characteristics (2)	Requirement (3)	Method of Test, Ref to (4)
i)	Vitamins of B-complex group mg/100 g, *Min*	5·0	See Note
ii)	Cyanocobalamine (Vitamin B_{12}), μg/100 g, *Min*	90	IS 7529 : 1975
iii)	β-Carotene, mg/100 g, *Min*	140·0	IS 5886 : 1970
iv)	Phosphorus (P) mg/100 g, *Max*	950·0	6 of IS 7874 (Part 2) : 1975
v)	Iron (Fe), mg/100 g, *Max*	120	7 of IS 7874 (Part 2) : 1975
vi)	Zinc (Zn), mg/100 g, *Max*	45·0	IS 7874 (Part 2) : 1975
vii)	Potassium (K), mg/100 g, *Max*	1 450	IS 9497 : 1980

NOTE — The vitamins of the B-complex group namely thiamine (Vitamin B_1), riboflavin (Vitamin B_2), nicotinic acid (Vitamin B_3) and pyridoxine (Vitamin B_6) shall be estimated individually according to the methods given in IS 5398 : 1969, IS 5399 : 1969, IS 5400, 1969 and IS 7530 : 1975, respectively and their total shall conform to the requirement given under col 3.

8.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070 : 1977) shall be used where the use of water as a reagent is intended.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

8.2.1 *Acceptance Tests*

Acceptance tests shall be carried out for the requirements given in Table 1.

8.2.2 *Type Tests*

Type tests shall be carried out for the requirements given in **4.4** and Table 2.

ANNEX A

(*Clause* 2.1)

LIST OF REFERRED INDIAN STANDARDS

IS No.	*Title*
1070 : 1977	Specification for water for general laboratory use (*second revision*)
1679 : 1960	Specification for sugar used in food preservation industry
5398 : 1969	Methods for estimation of thiamine (Vitamin B_1) in foodstuffs
5399 : 1969	Methods for estimation of riboflavin (Vitamin B_2) in foodstuffs
5400 : 1969	Methods for estimation of nicotinic acid (niacin) in foodstuffs
5401 : 1969	Method of detection and estimation of coliform bacteria in foodstuffs
5402 : 1969	Method for standard plate count of bacteria in foodstuffs
5886 : 1970	Methods for estimation of carotene and Vitamin A (retinol) in foodstuffs
5887 (Part 1) : 1976	Methods for detection of bacteria responsible for food poisoning: Part 1 Isolation identification and enumeration of *Escherichia coli* (*first revision*)
5887 (Part 3) : 1976	Methods for detection of bacteria responsible for food poisoning : Part 3 Isolation and identification of *Salmonella* and *Shigella* (*first revision*)
7529 : 1975	Method of estimation of vitamin B_{12} in foodstuffs
7530 : 1975	Method for estimation of pyridoxine (Vitamin B_6) in foodstuffs
7874 (Part 1) : 1975	Methods of test for animal feeds and feeding stuffs : Part 1 General methods
7874 (Part 2) : 1975	Methods of test for animal feeds and feeding stuffs : Part 2 Minerals and trace elements
7874 (Part 3) : 1975	Methods of test for animal feeds and feeding stuffs : Part 3 Microbiological methods
9497 : 1980	Method for determination of sodium and potassium (flame photometric)
9808 : 1981	Specification for fish protein concentrate

ANNEX B

(*Clause* 4.4)

BIOASSAY PROCEDURE TO TEST TOXIC FORMS OF ALGAE

B-1 PROCEDURE

B-1.1 Test Organism Used

Scenedesmus acutus (green algae)

B-1.2 Method of Raising Test Culture

Genetically pure strain of *Scenedesmus acutus* maintained as stock culture on agar slants with the medium as given in **B-1.3** and periodically subcultured (every 30 days).

Liquid cultures of *Scendesmus* is raised from the stock in 250 ml Erlenmeyer flasks in the same medium (each flask to contain 200 ml). The flasks are placed in rotary shaker (30-40 rpm), at temperature (25-28°C) with illumination provided by tubelights 4-5 K lux. The growth rate is monitored as optical density at 560 nm and the algal biomass expressed as mg dry algae/100 ml culture read from standard growth curve (0·1 OD=100 mg dry algae).

B-1.3 Nutrient Medium

Following standard medium is used for growing *Scenedesmus* :

$(NH_4)_2SO_4$	20 mg/l
K_2CO_3	25 mg/l
Na_3Po_4	25 mg/l
NaCl (Crude sea salt)	25 mg/l
$MgSO_4\ 7H_2O$	10 mg/l
$FeSO_4$	2 mg/l

B-1.4 Testing *Spirulina* Cultures

Cultures are drawn from the culture basins and filtered in 60-80 micron Nylon mesh to remove the *Spirulina* cells and the filtrate is centrifuged at 1 000 rpm for 10 minutes to concentrate other algal forms present in the filtrate. Ten ml of the sedimented filtrate is used in the test for toxicity by adding to the Erlenmeyer flask containing growing *Scenedesmus* cultures at 0·1 OD.

Six replicates of control (*Scenedesmus* alone) and experimental (*Scenedesmus* + 10 ml sedimented filtrate) were grown in flasks (**B-1.2**) and growth rate compared for a period of 5 days. Measurements of growth are recorded at OD every day. Reduction of growth of *Scenedesmus* culture by more than 10 percent as compared to control indicates inhibition of algal growth which is attributable to toxic algae being present in cultures.

ANNEX C

(*Clause* 6.1.1)

INSTRUCTIONS FOR USE AND STORAGE OF ALGA *SPIRULINA*, FEED GRADE

1 The material shall be kept sealed until use.

2 The material may be stored up to one year from date of manufacture.

3 If *Spirulina* is used as a replacement to the conventional protein sources, the recommended level of use is up to 20 percent of the feed protein requirements.

4 If *Spirulina* is used as an additive, the recommended level of use is 5 percent.

ANNEX D

(*Clause* 7.1)

SAMPLING OF FEED GRADE ALGA

D-1 GENERAL REQUIREMENTS OF SAMPLING

D-1.0 In drawing, preparing, storing and handling samples, the following precautions and directions shall be observed.

D-1.1 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

D-1.2 The sampling instrument shall be clean and dry when used.

D-1.3 Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers being sampled from adventitious contamination.

D-1.4 Samples shall be placed in clean, free from odour and dry glass containers. The containers shall be of such a size that they are almost completely filled by the sample.

D-1.5 Each container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name of the manufacturer and other important particulars of the consignment.

D-1.6 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

D-1.7 Sampling shall be done by a person agreed to between the purchaser and the supplier and in the presence of the purchaser (or his representative) and the supplier (or his representative).

D-2 SCALE OF SAMPLING

D-2.1 Lot

All the containers in a consignment belonging to the same batch manufacture shall constitute a lot.

D-2.2 The number containers to be tested from the lot shall depend on the size of the lot and shall be in accordance with Table 3.

Table 3 Scale of Sampling

Number of Containers in the Lot	Total Number of Containers to be Selected
(*N*)	(*n*)
(1)	(2)
Up to 50	3
51 ,, 200	4
201 ,, 400	5
401 ,, 650	6
651 and over	7

N being the number of containers in the lot and *n* the number of containers to be selected (*see* Table 3). Every container thus counted shall be withdrawn to give the requisite number of containers in the sample.

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